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Ultra-low oxygen refrigerated storage of 'rio red' grapefruit: fungistatic activity and fruit quality

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Abstract

Green mold development during refrigerated storage was suppressed, but not eradicated, by reducing the oxygen (O_2) concentration of the storage atmosphere from 21 to 0.10–0.05 kPa (ULO). The flavor and external appearance of grapefruit stored for 21 days at 14 °C under ULO, and 14 additional days in air at 23 °C was rated acceptable yet inferior to grapefruit stored similarly in air. A rind disorder developed as a post-hypoxic injury, especially on early-season, degreened grapefruit. The disorder was associated with an ULO-related increase in fruit acetaldehyde concentration. ULO may provide non-chemical suppression of green mold inside refrigerated marine containers en route to distant markets and postharvest insect control, but conditions must be optimized to avoid deleterious effects on fruit market quality. Published by Elsevier Science B.V.

Keywords: Citrus; Decay; Postharvest; Green mold; Controlled atmosphere; Storage; Quality

1. Introduction

Green mold, a major disease of citrus fruit, develops before or after fruit is harvested. The disease develops when spores of *Penicillium digitatum* (Pers.:Fr.) Sacc. infect minor wounds in fruit epidermal tissue (Brown and Eckert, 1988; Snowden, 1990). Commercial decay control measures include careful handling to minimize injuries, refrigerated storage after harvest, and pre or postharvest fungicides. In spite of these control measures, green mold development during shipping and transport remains a major cause of economic loss during marketing. Storage of grapefruit (*Citrus paradisi* Macf.) in air at the recommended temperature of 10–15 °C will suppress but not eliminate growth and sporulation of *P. digitatum*. Grapefruit are not usually stored at temperatures cooler than 10 °C because of susceptibility to chilling injury. Resistance of *P. digitatum* to fungicides such as benzimidazoles and others has been reported (Brown and Eckert, 1988).

The storage life of fresh grapefruit is limited by rind breakdown and decay to ≈ 2 weeks (Harden-

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burg et al., 1986). Market quality deterioration during storage is a product of fruit as well as pathogen physiological processes, such as respiration, transpiration, and altered resistance mechanisms. The atmosphere surrounding the fruit during refrigerated storage has been shown to beneficially alter these critical physiological processes (El-Goorani and Sommer, 1981; Sommer, 1985). Stahl and Cain (1937), Scholz et al. (1960), Harding (1969), and Seberry and Hall (1970) concluded that refrigerated (10 or 12 °C) storage under low (1–16 kPa) oxygen (O_2), with or without elevated concentrations of carbon dioxide (CO_2), did not effectively extend grapefruit storage (>10 weeks) to meet 'out of season' market demand, and demonstrated that CO_2 in excess of 20 kPa adversely affected rind integrity and increased the incidence of decay upon removal from controlled atmosphere (CA). Extended exposure to low O_2 also resulted in off-flavors and was associated with anaerobic metabolites.

Marketing opportunities for citrus in the Far East have created a need for extending grapefruit storage life by three rather than 10 weeks to permit marine container transport to distant markets. Decay development during surface transport is currently a major constraint to realizing these expanded market opportunities. Phytosanitary restrictions for quarantined insects also potentially limit market opportunities, and CA storage has been identified as a potential commodity quarantine treatment (Ke and Kader, 1991). Storage under atmospheres containing <1 kPa O_2, referred to as ultra-low O_2 (ULO), has been shown to suppress development of pathogenic fungi (Barkai-Golan, 1990) and kill insect pests (Ke and Kader, 1991). The objectives of the present research were to assess the extent to which refrigerated storage under ULO (<1 kPa) can suppress the growth of *P. digitatum* during short-term (~ 3 weeks) storage of grapefruit, and to assess fruit quality after refrigerated storage in ULO. The storage temperatures selected for research reflect those potentially efficacious for insect quarantine treatments (Ke and Kader, 1991).

2. Materials and methods

The fungistatic potential of ULO refrigerated storage was evaluated in replicated Experiments 1 and 2 conducted during the 1996 citrus season. Quality attributes of grapefruit after cold-storage in refrigerated ULO were evaluated in replicated Experiments 3, 4 and 5 conducted between 1995 and 1998. The 'Rio Red' grapefruit used in all experiments was grown on sour orange rootstock and acquired on the day of harvest from Texas Rio Grande Valley packing sheds (Experiments 1 and 4) or from an experimental grove at the USDA-ARS Subtropical Agricultural Research Center in Weslaco, TX (Experiments 2, 3 and 5). Fruit acquired from commercial packing sheds were waxed with Sta-Fresh 590 HS (FMC Corp., Lakeland, FL) containing a fungicide (Sodium orthophenyl-phenate (SOPP) or Thiabendazole (TBZ)) and transported in an enclosed van (15 min transit) to the ARS research laboratory in Weslaco, TX. Fruit harvested from the experimental grove were washed and dried by hand, and then waxed with Citrus Lustre 402 (Elf Atochem) without fungicide in a research scale, drip-style waxer.

The storage atmospheres utilized in all experiments were generated by a mixing board using liquid nitrogen, and compressed cylinders of CO_2 or air. Atmospheres containing desired amounts of O_2 and or CO_2 were delivered via nylon Legris™ (Legris Inc., Mesa, AZ) tubing (0.6 cm o.d.) to 19 l plastic storage buckets located inside a temperature controlled chamber. The gas was circulated through a gas-tight jar containing water that was located inside the walk-in cooler, upstream from the buckets. A small length of glass capillary tubing located between the hydrator jar and the bucket regulated flow inside each bucket at 250 ml min^{-1}. Each bucket contained 10 or 15 grapefruit. A 2-m piece of tubing attached to a barbed fitting opposite each bucket inlet was used to measure flow and atmosphere composition exiting the bucket. The concentration of O_2 and CO_2 at the mixing board and exiting each bucket was monitored twice daily with a portable combination

O_2 and CO_2 analyzer (PBI-Dansensor, Denmark). Gas concentrations are expressed in Pascals (Pa) as partial pressures according to Banks et al. (1995). Atmospheric pressure at sea level was 100 kPa, so 21% O_2 at sea level was equal to a partial pressure of 21 kPa. Temperature inside the walk-in cooler was continuously monitored with an Omega RD-Temp-XT data logger (Omega Engineering Inc. Stamford, CT). After cold storage, fruit were transferred to half standard cardboard cartons and stored in air at 23 °C and 90% relative humidity for 3 (Experiment 3), 5 (Experiments 1 and 2), or 14 days (Experiment 4).

A modified method of Eckert and Brown (1986) was used to inoculate grapefruit in decay control Experiments 1 and 2 with a spore suspension of *P. digitatum* containing 10^6 spores per ml (Shellie and Skaria, 1998). Spores were originally isolated from 'Rio Red' grapefruit and cultures of *P. digitatum* were maintained on potato-dextrose agar. Spore suspensions were prepared to count using a hemacytometer under a light microscope, and final volume was adjusted with distilled water. A drop of Tween-20 was added for uniform suspension, and the solution was gently agitated prior to inoculation on a shaker at 23 °C for 3 h. Grapefruit were inoculated by making a 6-mm-deep puncture into the flavedo of the fruit equatorial region with a 1-mm wide, sterile nail, and pipetting 10 (Experiment 2) or 20 (Experiment 1) μl of spore suspension into the wound. The site of inoculation was demarcated on the flavedo tissue with a permanent ink marker. Decay progression was quantified by measuring (in cm) the diameter of the concentric, water soaked lesion emanating from the inoculation site. Fruit lesion diameter was measured upon removal from cold storage and again after 5 days storage in air at 23 °C. Lesion growth during storage at 23 °C was calculated by subtracting lesion diameter after cold storage from lesion diameter after storage at 23 °C.

The quality attributes evaluated in Experiments 3, 4, and 5 included fruit weight, flavedo color, decay severity, flavor, external appearance, soluble solids concentration, and titratable acidity. Quality attributes were analyzed as the average value of measurements made per treatment group on 30 (Experiments 3 and 4) or 45 (Experiment 5) fruit, or ratings from 20 untrained panelists. The weight, flavedo color, titratable acidity, and soluble solids concentration of ten fruit on each day of harvest were evaluated to verify fruit maturity at each experimental replication. Treatment atmospheres inside each bucket were established 24 h after fruit were placed into cold storage.

Each fruit in Experiments 3, 4, and 5 was numbered and weighed prior to cold storage and again after storage in air at 23 °C. Percent weight loss was calculated by dividing the difference between pre cold-storage weight and post-23 °C storage weight by pre cold-storage weight and multiplying by 100. Three, 2-cm diameter circles were drawn along the equatorial surface of each fruit, and flavedo color was measured inside the demarcated circles prior to cold storage and after storage in air at 23 °C with a chromameter (model CR-200; Minolta Corp., Ramsey, NJ in the L, C^*, $h°$ color scale (L = darkness, C^* = chroma or color intensity, and $h°$ = hue) and recorded as an average of three readings. Flavedo color change was calculated as a ratio of pre cold-storage to post-23 °C storage for values of L^*, C^* and $h°$. Fruit were rated for severity of decay or rind disorders and external appearance after cold storage and after storage in air at 23 °C. Decay and rind disorders were rated on a five-point scale based on percent affected fruit surface with 0, 1, 2, 3, or 4 signifying $\leqslant 0$, 15, 50, 85, or 100%. External appearance was rated on a five point scale with 0, 1, 2, 3, or 4 signifying marketability as excellent, good, questionable, poor, or unmarketable. An untrained, 20 member preference panel rated four representative fruit from each treatment group for flavor and external appearance by placing a mark on a 9 cm, continuous line scale. Two fruit from each treatment group were sectioned into bite size pieces or displayed at independently randomized stations. The line scale was labeled 'dislike extremely' at the left and 'like extremely' at the right. Preferences were quantified by measuring the distance in cm from the extreme left of the line scale to the indicated preference mark (ASTM, 1968). The remaining intact fruit from each treatment group were juiced with an electric rotary juicer, and soluble solids concentration and titratable acidity determined as described by Shellie and Mangan (1993).

2.1. Fungistatic potential of ULO refrigerated storage: experiments 1 and 2

2.1.1. Experiment 1: effect of O_2 concentration on decay

Two hundred and twenty waxed fruit (with fungicide) acquired from a packing shed four times between the months of February and April 1997 were stored for 3, 7, 14, or 21 days at 16 °C in 0.05, 0.10, 0.25, 0.50, or 1.0 kPa of O_2 or in air for 3 or 7 days. Fruit pulp temperature at inoculation was ~21 °C and fruit were immediately transferred to 16 °C. Data were analyzed as the average of ten fruit with packing shed acquisitions considered replications. A linear model for predicting lesion diameter after cold storage under each ULO atmosphere was developed from linear regression. The slope of each line was tested for difference from the slope of the line predicting lesion diameter after storage in 0.05 kPa O_2 using SAS general linear model procedure. Lesion growth during 5 days of storage in air at 23 °C was analyzed in a factorial ANOVA with atmosphere and duration of prior cold storage as main effects. Mean values for significant main effects were separated using Duncans multiple range test.

2.1.2. Experiment 2: effect of wax, atmosphere and storage temperature on growth of green mold

One hundred and sixty waxed (without fungicide) grapefruit acquired in each of two harvests during the month of December 1996 were stored in 0.05 kPa O_2 or air at 14 or 18 °C for 14 (first harvest) or 7 (second harvest) days. A waxed and unwaxed, non-inoculated check was evaluated to verify inoculum growth. Fruit were inoculated after 24 h of storage in air at 14 or 18 °C, and immediately placed back into cold storage under 0.05 kPa O_2 or air. Data from each harvest were analyzed separately with ten inoculated fruit in each treatment group considered replications. A split-plot analysis of variance (ANOVA) was used to analyze the data with storage temperature as the main split and storage atmosphere and wax as main effects. Mean separation for significant main effects was accomplished using a t-test.

2.2. Quality of grapefruit after refrigerated ULO storage: experiments 3, 4, and 5

2.2.1. Experiment 3: effect of ULO with or without 20 kPa CO_2 on fruit quality

One thousand waxed (without fungicide) grapefruit harvested three times during the months of February and March 1996 were stored for 14 or 21 days at 10, 12, or 14 °C in 0.05, or 0.10 kPa O_2 with or without 20 kPa CO_2, or in air. Data for quality attributes were analyzed in a factorial ANOVA with atmosphere, storage temperature, storage duration, and replication as main effects. When a main effect was significant, mean values were separated by Duncans multiple range test.

2.2.2. Experiment 4: effect of 21 days of 0.05 kPa O_2 storage at 14 °C on quality of waxed fruit

Seventy waxed (with fungicide) grapefruit acquired six times between the months of January and April in 1996–1998 were stored for 21 days at 14 °C in 0.05 kPa O_2 or air. Data were analyzed in a factorial ANOVA with atmosphere (0.05 kPa O_2 or air), year (three levels), and replication (six levels) as main effects. Samples of five fruit stored under 0.05 kPa O_2 or air from experimental replications one, three, and six in 1998 were sent to the USDA-ARS laboratory in Winter Haven, FL for quantitative analysis of flavor volatiles as described by Baldwin et al. (1995). Results of volatile analyses were analyzed as an average value of five fruit in an ANOVA with storage atmosphere and replication as main effects.

2.2.3. Experiment 5: effect of fruit maturity and degreening on fruit quality after 21 days of storage in 0.05 kPa O_2 at 14 °C

One hundred and fifty grapefruit harvested three times during December, 1998 were transported (enclosed van, 15 min transit) to a commercial packing shed, and stored for 24 h inside a commercial degreening room together with a commercial load of grapefruit. Temperature and ethylene concentration inside the degreening room was not monitored but assumed to be rep-

resentative of standard industry practices (25 °C, ethylene 1–10 μl^{-1}, 90–95% relative humidity). After exposure to ethylene, fruit were transported back to the ARS research laboratory, waxed (without a fungicide) with the remaining harvested fruit, and stored at 14 °C in 0.05 or air for 21 days. Ratings were analyzed as the mean of 45 fruit per treatment group in a factorial ANOVA with degreening, atmosphere, and replication as main effects.

3. Results

3.1. Fungistatic potential of ULO refrigerated storage on grapefruit

3.1.1. Experiment 1: effect of O_2 concentration on decay

Growth of green mold during storage at 16 °C increased as the concentration of O_2 in the storage atmosphere increased from 0.05 to 1.00 kPa (Table 1). The slope of the line predicting growth of lesion diameter during storage increased from 0.111 when fruit were stored in 0.05 kPa O_2 to 0.389 when fruit were stored in 1.00 kPa O_2. Slope estimates for storage under 0.05 or 0.10 kPa O_2 were similar, suggesting that O_2 concentration can vary from 0.05 to 0.10 kPa without compromising decay suppression. Slope estimates increased as the concentration of O_2 in the storage atmosphere equaled or exceeded 0.25 kPa. Fruit stored for 7 days under 1.0 or 0.05 kPa O_2 had 40 or 97% smaller (3.6 and 0.2 cm) lesion diameters than fruit stored for the same period of time in air (6.0 cm).

The number of days grapefruit were cold-stored under 0.05 kPa O_2 had a residual influence on the magnitude of lesion growth during subsequent storage in air at 23 °C (Table 2). Lesion growth at 23 °C was least when fruit was cold-stored 21 days under ULO, and greatest after 7 days of ULO cold storage. Lesion growth at 23 °C was similar for fruit cold-stored under ULO for 3 or 14 days. Fruit cold-stored in air for 3 or 7 days had lesion growth at 23 °C of 11.3 cm.

3.1.2. Experiment 2: effect of wax, atmosphere and storage temperature on growth of green mold

Inoculated fruit removed from 0.05 kPa O_2 cold storage had no visible decay lesions whereas fruit cold-stored in air had well-developed, concentric lesions (Table 3). Decay lesions developed on fruit cold-stored in 0.05 kPa O_2 after five additional days of storage in air at 23 °C. Grapefruit cold-stored in air for 7 days at 18 °C developed 88% larger lesions than fruit stored in air at 14 °C. However, fruit stored in air for 14 days (second harvest) at 14 or 18 °C had similar sized lesions. Waxing

Table 1
Slope estimates and average green mold lesion diameter on grapefruit stored at 16 °C in an atmosphere of 1.00, 0.50, 0.25, 0.10, or 0.05 kPa O_2 (balance nitrogen) for 3, 7, 14, or 21 days, or air for 3 or 7 days (Experiment 1)

Storage atmosphere	Lesion diameter (cm)				Intercept	Slope	Probability for slope estimates[a]	
	3 days	7 days	14 days	21 days			O_2 (0.05 kPa)	O_2 (0.25 kPa)
Air	0.60	6.00	NE	NE	NE	NE	NE	NE
1.00 kPa O_2	0.21	3.61	4.06	8.44	−0.23	0.389	0.001**	0.001**
0.50 kPa O_2	0.05	3.06	4.60	4.89	−0.23	0.286	0.001**	0.05*
0.25 kPa O_2	0.13	1.63	2.50	4.34	−0.23	0.213	0.008**	
0.10 kPa O_2	0.00	0.60	1.70	2.31	−0.23	0.125	0.721	0.02*
0.05 kPa O_2	0.00	0.18	1.81	1.94	−0.23	0.111		0.008**

Fruit were waxed with a fungicide and inoculated with 20 μl of a 10^6 spore solution of *P. digitatum*. Data were analyzed as the average of ten fruit in each of four replications. NE, not evaluated.

[a] Probability that a slope is significantly different than the slope that predicts lesion diameter for fruit stored under 0.05 or 0.25 kPa O_2, respectively.

* $P \leqslant 0.05$.

** $P \leqslant 0.01$.

Table 2
Mean values and mean squares for lesion growth during 5 days in air at 23 °C (Experiment 1)

Source	Lesion growth of ULO stored fruit after 5 days at 23 °C			
	df	Low O_2	df	Air
Mean values (cm)				
Days cold storage				
3		6.6a		10.9a
7		9.4b		11.7a
14		4.4ac		NE
21		3.3c		NE
Mean squares				
Storage atmosphere (A)	4	15.40		
Days cold storage (S)	3	145.90**	1	1.29
$A \times S$	12	5.33		
Error	60	15.44	6	12.19

Fruit were waxed with a fungicide, inoculated with 20 μl of a 10^6 spore solution of *P. digitatum*, and stored at 16 °C in 1.00, 0.50, 0.25, 0.10, or 0.05 kPa O_2 (balance nitrogen) for 3, 7, 14, or 21 days, or in air for 3 or 7 days. Data were analyzed as the average of ten fruit in each of four replications. Similar letters within a column indicate no significant difference at $P \leqslant 0.05$. NE, not evaluated.

** $P \leqslant 0.01$.

(without fungicide) had no effect on lesion diameter. Grapefruit not inoculated with *P. digitatum* had no visible symptoms of green mold after 7 or 14 days of storage (data not shown).

3.2. Grapefruit quality after refrigerated ULO storage

3.2.1. Experiment 3: effect of ULO with or without 20 kPa CO_2 on fruit quality

Grapefruit stored under ULO were rated inferior in flavor to grapefruit stored in air (Table 4). The flavor of grapefruit stored in 0.10 kPa O_2 was rated as similar to that of grapefruit stored in 0.05 O_2. The flavor of grapefruit stored in 0.10 kPa O_2 with 20 kPa CO_2 were rated inferior to that of grapefruit stored under 0.10 kPa O_2 (balance nitrogen). Fruit stored under CA lost more weight than fruit stored in air (Table 4). Storage temperature and storage duration also influenced percent fruit weight loss, with fruit stored at higher temperatures for a longer duration losing the greatest amount of weight (data not shown). No significant interactions were observed between storage atmosphere, temperature, and storage duration. Storage atmosphere had no effect on percent titratable acidity, soluble solids concentration, flavedo color or ratings or external appearance.

3.2.2. Experiment 4: effect of 21 days of 0.05 kPa O_2 storage at 14 °C on quality of waxed fruit

In 1996 and 1998, panelists rated the flavor of grapefruit stored in 0.05 kPa O_2 as inferior to that of grapefruit stored in air (Table 5). However, in 1997, panelists rated the flavor of grapefruit stored in 0.05 kPa O_2 as superior to that of grapefruit stored in air. The external appearance of grapefruit stored in 0.05 kPa O_2 was consistently rated each year by panelists as inferior to that of grapefruit stored in air. Fruit stored under 0.05 kPa O_2 also lost more weight than fruit stored in air. Storage atmosphere had no influence on percent titratable acidity, soluble solids concentration, or flavedo color.

The concentrations of nine volatile components were greater in grapefruit previously stored for 21 days in 0.05 kPa O_2 compared to grapefruit stored for a similar amount of time in air (Fig. 1). The concentration of ethyl acetate and methyl butyrate in fruit cold-stored under ULO was greater than 3-fold higher 14 days after transfer from ULO to air relative to fruit cold-stored in air. The

concentration of *trans*-2-hexenol, ethanol, and acetaldehyde were also 50% or more higher relative to fruit cold-stored in air. The concentrations of eight other volatiles (methanol, hexanol, *cis*-3-hexenol, α-terpineol, octanal, hexanal, decanal, and linalool) were similar 14 days after transfer from ULO to fruit that had been cold-stored in air.

3.2.3. Experiment 5: effect of fruit maturity and degreening on fruit quality after 21 days of storage in 0.05 kPa O_2 at 14 °C

Storage in 0.05 kPa O_2 for 21 days at 14 °C adversely affected fruit external appearance and severity of rind disorder (Table 6). The external appearance of degreened grapefruit was rated similar to non-degreened fruit, however, degreened fruit developed more severe rind disorder. Rind disorder was not apparent upon removal of the fruit from ULO storage, but developed during 14 days of subsequent storage in air at 23 °C. The rind disorder appeared as a well delineated, dry, brown recessed area dispersed along the fruit surface, and was most prevalent on degreened fruit after storage in 0.05 kPa O_2.

4. Discussion

Freedom from decay and disorders, good external appearance and good flavor are principal market quality requirements of fresh grapefruit. Our results demonstrated that refrigerated storage in 0.05 or 0.10 kPa O_2 suppressed growth of *P. digitatum* and that growth resumed as the concentration of O_2 in the storage atmosphere increased

Table 3
Mean values and mean squares from ANOVA for lesion diameter of waxed and unwaxed grapefruit stored at 14 or 18 °C in air or 0.05 kPa O_2 (Experiment 2)

Source	df	Lesion diameter after cold storage		Growth in lesion diameter[a] during 5 days at 23 °C	
		7 days	14 days	7 days	14 days
Mean values (cm)					
Temperature					
14 °C	40 (20[b])	3.6a	11.8a	8.3a	5.7a
18 °C	40 (20)	6.8b	12.7a	10.3a	2.6a
Atmosphere					
0.05 kPa O_2	40 (20)	0.0a	0.0a	9.3	4.2
Air	40	10.4b	24.5b	NE	NE
Wax					
Yes	40 (20)	4.6a	15.0a	6.1a	5.3a
No	40 (20)	5.8a	9.5a	12.5a	3.1a
Mean squares					
Temperature (T)	1	33.60*	45.50	0.15	7.82
Error	2	1.97	28.25	34.64	36.16
Atmosphere (A)	1	402.1**	3064.79**	NE	NE
Wax (W)	1	5.47	45.50	42.69	20.02
$A \times W$	1	5.47	45.50	NE	NE
Error	6	36.65	14.06	7.55	6.80

Grapefruit were inoculated with 10 μl of a 10^6 spore solution of *P. digitatum*. Fruit were stored for 7 (first harvest) or 14 days (second harvest). Similar letters within a column indicate no significant difference at $P \leqslant 0.05$. NE, not evaluated.

[a] All fruit were previously cold-stored at 14 or 18 °C for 7 or 14 days under 0.05 kPa O_2.

[b] Sample size of fruit stored for five additional days in air at 23 °C.

* $P \leqslant 0.05$.

** $P \leqslant 0.01$.

Table 4
Mean values and mean squares from ANOVA for quality attributes of grapefruit after 14 or 21 days of storage in air or ULO (0.05 or 0.10 kPa O_2) with or without 20 kPa CO_2 at 10, 12, or 14 °C (Experiment 3)

Source	df	Flavor[a]	External appearance[a]	Weight (% loss)	Acidity (%)	SSC (%)
Mean values						
Atmosphere (kPa)						
Air		6.3a	6.5a	0.63a	0.802a	8.4a
0.10 O_2		5.6b	6.4a	0.95b	0.783a	8.4a
0.05 O_2		5.2bc	6.5a	0.90b	0.775a	8.2a
0.10 O_2+20 CO_2		4.9c	6.7a	0.93b	0.760a	8.2a
0.05 O_2+20 CO_2		4.8c	6.4a	0.93b	0.788a	8.2a
Mean squares						
Atmosphere (A)	4	6.07**	0.28	0.33**	0.003	0.47
Temperature (T)	2	0.69	0.30	0.61**	0.043**	0.81
Duration (D)	1	0.00	2.20	0.76**	0.000	0.49
$A \times T$	8	0.29	1.50	0.04	0.003	0.09
$A \times D$	4	0.98	0.45	0.03	0.007	0.06
$T \times D$	2	0.11	3.02	0.13	0.020	0.17
$A \times T \times D$	8	0.42	0.81	0.04	0.007	0.05
Replication	2	0.53	0.09	0.07	0.087**	0.70*
Error	58	0.75	1.06	0.06	0.007	0.15

Data were analyzed as the average of 30 fruit. Similar letters within a column indicate no significant difference at $P \leqslant 0.05$.

[a] Rating of grapefruit by untrained preference panelists after three additional days in air at 23 °C using a 9.5 cm line scale with 1 = dislike extremely and 9 = like extremely. Means are based on the average of 20 judges in each of three replications.

* $P \leqslant 0.05$.

** $P \leqslant 0.01$.

above 0.25 kPa. Our results with inoculated fruit support findings of Wells and Uota (1970) for *Alternaria tenuis*, *Fusarium roseum*, *Botrytis cinerea*, and *Cladosporium herbarum* grown on artificial medium. Growth of these fungi declined by 85% when the artificial medium was exposed to 1 kPa O_2, and ceased completely when exposed to anoxia. It remains unknown why *P. digitatum* fails to grow in the absence of molecular O_2, but effects on the cytochrome system of electron transport are suspected to be involved (Tabak and Bridge Cooke, 1968).

Sommer (1985) and Barkai-Golan (1990) suggested that refrigerated ULO storage reduced decay by directly suppressing pathogen growth and by indirectly maintaining resistance of the host to infection. They both highlighted the importance of low temperature in combination with ULO for optimum suppression. Results from this research show that inoculated fruit stored under ULO at 18 °C developed larger lesion diameters than fruit stored under ULO at 14 °C. Most pathogens have optimum growth rates at 25 °C (Sommer, 1985). Germination and growth of *P. digitatum* can be prevented by storage in air at 1 or 3 °C, respectively (Brown, 1922; Sommer, 1985). However, grapefruit become unmarketable when stored at these temperatures, and Scholz et al. (1960) have shown that storage under CA did not alleviate development of chilling injury symptoms. Sommer (1985) described disease lesion development as a sigmoidal curve, with the initial lag period as the time in which infection becomes established. Fungi are generally considered to be obligate aerobes, and O_2 concentrations of 3–4 kPa are restrictive to their growth (Brown, 1922; Tabak and Bridge Cooke, 1968; Sommer, 1985). Low temperature and/or reduced levels of O_2 in the storage environment during the initial lag phase of lesion development suppress decay by prolonging the initial period of infection. Our observations that storage in ULO for up to 14 days provided some residual suppression during subsequent storage in air at 23 °C most likely results from an inhibition of initial infection.

In our research, the external appearance of grapefruit stored under ULO was rated as acceptable (rated $\geqslant 5$ on a nine-point scale), yet inferior, in some years, to grapefruit stored in air. The inferior appearance of fruit stored under ULO in our research was described as 'aged' (data not shown). Fruit stored under ULO in our research lost more weight than fruit stored in air, and this could explain a more 'aged' appearance, because no rind injury was apparent on these fruit. Stahl and Cain (1937) reported that grapefruit stored in N_2 gas at 3 °C for 4 weeks, maintained a bright, firm appearance. And, Ke and Kader (1990) reported that oranges kept in ULO at 10 °C for 20 days and then transferred to air at 5 °C for 7 days had similar external and internal appearance scores as fruit stored in air. The acceptable, yet 'aged' appearance of fruit stored under ULO observed in our research could be because we stored our fruit at 14 instead of 10 or 3 °C, and because our subsequent storage temperature was warmer and more prolonged. In other words, our storage conditions permitted more degradation of the product than that of Stahl and Cain (1937), and Ke and Kader (1990). The higher storage temperatures evaluated in our research would be expected to result in higher rates of respiration and fruit water loss.

Ke and Kader (1990) reported that Valencia oranges stored at 10 °C in 0.02 kPa O_2 had a higher CO_2 production rate than oranges stored in 0.25 kPa O_2, and speculated this was due to anaerobic respiration. According to Biale (1954) the anaerobic compensation point (ACP) for oranges is 2.5–5 kPa O_2, so production of anaerobic metabolites would be expected to increase in fruit stored under ULO. Anaerobic metabolites, such as ethanol, acetaldehyde, and ethyl acetate, and the balance of available glycolytic substrates like sugars and acids, contribute to perceived citrus

Table 5
Mean values and mean squares from ANOVA for quality attributes of grapefruit after storage for 21 days at 14 °C in air or 0.05 kPa O_2 (Experiment 4)

Source	Flavor[a]	External appearance[a]	Weight (% loss)	Acidity (%)[b]	SSC (%)
Mean values					
1996					
0.05 O_2	5.1	6.2	3.0	0.84	9.4
Air	7.5	7.0	2.3	0.86	9.8
1997					
0.05 O_2	5.1	5.2	2.2	0.83	8.4
Air	4.5	5.6	1.6	0.86	8.7
1998					
0.05 O_2	5.6	5.8	NE	NE	NE
Air	6.3	6.0	NE	NE	NE
Mean squares					
Atmosphere (A)	6.98*	2.27*	2.48*	2.84	0.95
Year (Y)	7.49**	4.25**	3.61**	0.12	6.15*
$A \times Y$	6.70**	0.32	0.01	0.21	0.01
Replication	0.75	1.08	0.24	46.77**	2.05
Error	1.15	0.33	0.43	4.76	1.31

Data were collected over three sequential citrus seasons, with six harvests per year as replications. Data were analyzed as the average of 30 fruit.

[a] Rating of grapefruit by untrained preference panelists after 14 additional days in air at 23 °C using a 9.5 cm line scale with 1 = dislike extremely and 9 = like extremely. Means are based on the average of 20 judges in each of six replications.

[b] Mean square $\times 10^3$.

* $P \leqslant 0.05$.

** $P \leqslant 0.01$.

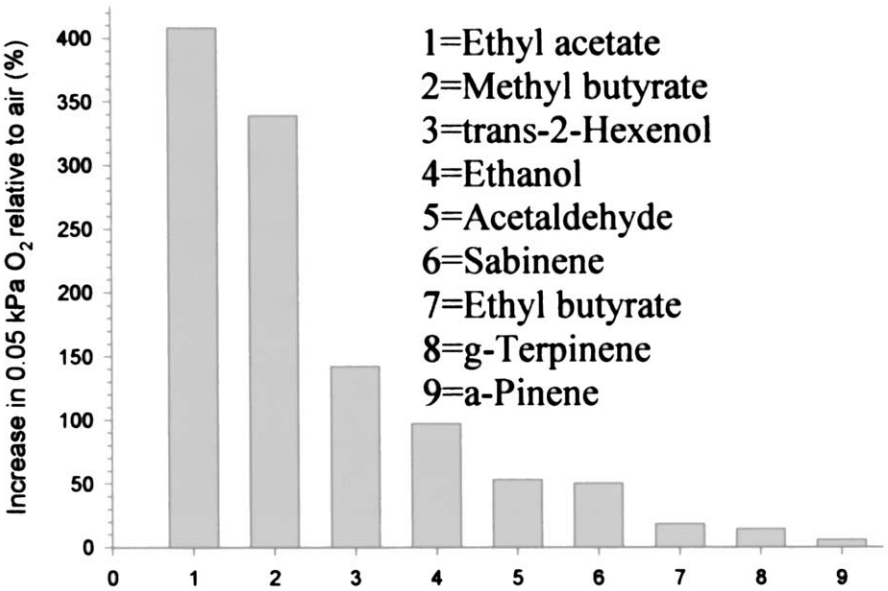

Fig. 1. Percent increase in nine volatile constituents of grapefruit stored for 21 days at 14 °C in 0.05 kPa O_2 (balance N_2) relative to grapefruit stored in air (Experiment 4). Data were analyzed as the average of five individual analyzes for each of five fruit in three experimental replications.

flavor. In our research, grapefruit stored under ULO had similar concentrations of soluble solids and titratable acidity as grapefruit stored in air, but an increase in nine out of 17 measured flavor volatiles. Our results support the findings of Scholz et al. (1960), and Ke and Kader (1990) who observed similar concentrations of soluble solids and titratable acidity in grapefruit stored in low O_2 (1–5 kPa) for 9 weeks at 5 °C or in 'Valencia' oranges stored under 0.5, 0.25, or 0.02 kPa O_2 at 0, 5, or 10 °C for 20 days, respectively. However, Davis et al. (1973) reported a correlation of 0.765 between increased concentration of ethanol and decreased soluble solids and acid in grapefruit stored at 4 °C in low (5–15 kPa) O_2 for 6 weeks, and concluded that sugar served as a major source for ethanol synthesis in grapefruit.

In our research, the flavor of grapefruit stored under ULO was rated acceptable (rated $\geqslant 5$ on a nine-point scale) yet inferior to grapefruit stored in air. These findings concur with that of Stahl and Cain (1937), Scholz et al. (1960), and Ke and Kader (1991). Our findings showed that grapefruit flavor was further adversely affected by addition of 20 kPa of CO_2 to the ULO atmosphere. Stahl and Cain (1937), and Scholz et al. (1960) reported a similar adverse response of grapefruit to storage in elevated levels of CO_2. Harding (1969), and Ke and Kader (1991) attributed the off-flavor of grapefruit stored 6 weeks at 12 °C under low (3–3.5 kPa) O_2 or 'Valencia' oranges stored under 0.5, 0.25, or 0.02 kPa O_2 at 0, 5, or 10 °C for 20 days to elevated concentrations of ethanol and acetaldehyde. Norman and Craft (1971) documented that concentrations of ethanol, methanol, acetaldehyde and ethyl acetate remained elevated 14 days after fruit were transferred from ULO to air at 20 °C. The increase in volatiles observed in our research support findings of Norman and Craft (1971).

The rind disorder we observed on early-season, degreened grapefruit developed after fruit were transferred from ULO to air at 23 °C. Other researchers have reported a similar disorder. Nelson (1933) described 'Brown spot' or 'Storage spot' in citrus stored in N_2 for 7 days at 10 °C as a rind disorder that developed 3–5 days after fruit are transferred from ULO to air and began as a colorless, collapse of active parenchymal cells surrounding oil glands. These collapsed cells desic-

cated, eventually causing a collapse of oil glands, and the entire depressed area turned dark brown. Harding (1969) reported a rind disorder called 'peteca' that developed on grapefruit stored for 75 days at 12 °C under low (3.5 kPa) O_2 and elevated (1.5–2.5 kPa) CO_2. Scholz et al. (1960) reported that many grapefruit stored at 50 °C for 6 weeks developed brown, sunken areas, and Norman and Craft (1971) reported that oranges held in N_2 for $\geqslant 3$ days at 20 °C developed brown spots after the fruit were returned to air. The rind injury observed in our research was similar to that described by Nelson (1933), and Norman and Craft (1971), and can be classified as a 'post-hypoxic' injury.

Fruit acetaldehyde concentration most likely plays an important role in post-hypoxic injury. Onset of rind injury was shown to correspond temporally with elevated concentrations of acetaldehyde and ethyl acetate in the fruit (Norman and Craft, 1971). Norman and Craft (1971) documented that ethanol and methanol concentration increased while oranges were stored in N_2 for 5 days at 20 °C. However, acetaldehyde and ethyl acetate concentration did not greatly increase until the oranges were transferred from N_2 storage to air at 20 °C. The redox ratio of NAD is known to affect activity of alcohol and malate dehydrogenase (ADH, MDH), with a higher redox ratio associated with transition toward anaerobiosis. The NADH-NAD redox ratio of oranges incubated under N_2 at 34 °C for 18 h was double that of oranges stored at a similar temperature in air, and corresponded little to accumulation of acetaldehyde under ULO. However, when NADH oxidation became quickly re-established upon transfer of fruit to air, the accumulated ethanol was rapidly oxidized by ADH (Chervin et al., 1996) and acetaldehyde concentration in the fruit increased dramatically (Norman and Craft, 1971). Further evidence of involvement of acetaldehyde in this disorder is that 'brown spot' or 'storage spot' was induced by exposure of fruit in air to vapors of acetaldehyde (Nelson, 1933). 'Valencia' oranges stored in ULO at 10 °C for up to 20 days and then transferred to air at 5 °C for 7 days did not develop post-anoxic rind injury (Ke and Kader, 1990), suggesting that one possibility of alleviating this disorder may be to transfer fruit to air at 10 instead of 23 °C.

Ethylene has been shown to stimulate respiration in citrus and enhance fruit maturation (Bruemmer, 1986). The NAD redox ratio has also been shown to increase with fruit maturation. Mature grapefruit stored in air containing 20 ppm ethylene contained 7–10 times more ethanol after 12 weeks at 15 °C than control fruit stored without ethylene. The acetaldehyde concentration of fruit treated with ethylene was about three times higher. Malate values were lowest in samples with the highest ethanol concentration, suggesting that ethylene promoted metabolism of malate to ethanol. Nelson (1933) has shown that early season grapefruit are more susceptible to rind injury than late season fruit. The high incidence of rind injury we observed in early season, degreened grapefruit could be attributed to the role of ethylene in stimulating fruit respiration.

Table 6
Mean values and mean squares from ANOVA for quality ratings of degreened and non-degreened grapefruit after storage for 21 days at 14 °C in air or 0.05 kPa O_2 and 14 additional days of storage in air at 23 °C and 90% relative humidity (Experiment 5)

Source	External appearance[a]	Rind disorder[b]
Mean values		
Air		
Degreened	1.7a	0.07
Non-degreened	1.6a	0.16
0.05 kPa		
Degreened	2.8b	0.45
Non-degreened	2.0b	0.27
Mean squares		
Atmosphere (A)	1.86*	0.18**
Degreening (D)	0.76	0.01
$A \times D$	0.47	0.06*
Replication	4.44**	0.06*

Fruit were harvested three times in December of 1998, and data were analyzed as an average of 45 fruit. Similar letters within a column indicate no significant difference at $P \leqslant 0.05$.

[a] Five-point rating scale with 0, 1, 2, 3, or 4 signifying marketability as excellent, good, questionable, poor, or unmarketable, respectively.

[b] Five-point rating scale with 0, 1, 2, 3, or 4 signifying $\leqslant 0$, $\leqslant 15$, $\leqslant 50$, $\leqslant 85$, or $\leqslant 100\%$ of the fruit surface.

* $P \leqslant 0.05$.

** $P \leqslant 0.01$.

5. Conclusion

Beneficial suppression of green mold can be accomplished by reducing the O_2 concentration of the storage atmosphere from 21 to 0.10–0.05 kPa. If fruit are stored under ULO for 14 or 21 days, residual suppression may be apparent after fruit are removed from ULO and stored in air. Establishment of an ULO atmosphere inside a refrigerated marine container during a 21 days voyage to distant markets could provide beneficial suppression of green mold. A reduction in market quality (flavor and rind disorders) became apparent after removal from ULO storage, especially in early season fruit that had been degreened prior to ULO storage. Even though the flavor of grapefruit stored under ULO was rated inferior to grapefruit stored in air, the flavor of the grapefruit stored under ULO was rated as acceptable. Knowing the tolerance of grapefruit to short-term storage in O_2 at or below 1 kPa may also be useful for developing non-chemical quarantine treatments for postharvest insect control.

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